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Persistence of Polybrominated Biphenyls (PBB) in Human Post-Mortem Tissue

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Polybrominated biphenyl (PBB), a fire retardant, was accidentally substituted for an animal feed supplement in Michigan 10 years ago. This led to widespread livestock contamination and ultimately to contamination of virtually every human residing in the State at that time. In order to evaluate the extent of PBB persistence and distribution in human tissues 10 years after this accidental poisoning, a study was conducted on a series of autopsy cases from the Grand Rapids ("high" exposure) area of the State. No attempt was made to relate cause of death to PBB exposure or tissue concentration.

Samples of 196 tissues from 15 subjects were analyzed for PBB content, and levels were determined by measurement of the hexabromobiphenyl peak using electron capture gas chromatography. Only 4 of the 196 samples analyzed did not have PBB concentration above the limit of detection (0.5 ng/g).

As expected, fat and fat-rich tissue had the highest PBB concentration. Perirenal fat had the highest mean concentration (475 ng/g). Adrenal, atheromatus aorta and thymus had mean concentrations about half that of perirenal fat; all other tissues had mean concentrations one-tenth or less of perirenal fat.

The results document that PBB is still present in human tissue and that PBB was distributed in all tissues examined. The PBB fat elimination half-time was estimated to be at least 7.8 years. If this is approximately correct, PBB will persist in tissues throughout the lifetime of humans so contaminated.

Introduction

Ten years ago in Michigan, Firemaster BP-6, a commercial fire retardant composed of a mixture of polybrominated biphenyls (PBB), was accidentally substituted for Nutrimaster, magnesium oxide for use in animal feed as a supplement. It has been estimated that one to two tons of PBB was erroneously mixed into animal feed (*1*). More than 90% of the human population (*2*) residing in Michigan at that time eventually was exposed to PBB-contaminated meat and dairy products.

In the years following this mishap, studies conducted in animals have demonstrated that PBB may be hepatotoxic, neurotoxic, immunotoxic, and carcinogenic (*3–7*). Since PBB is structurally similar to polychlorinated biphenyl (PCB), it is known that PBB would be highly lipid-soluble, highly persistent in the organism, and highly resistant to biotransformation. Miceli and Marks (*8*) have demonstrated body-wide PBB distribution and long-term persistence of PBB in body tissue of rats. Indeed, they calculated that PBB would not be expected to be eliminated during the animal's lifetime.

PBB concentrations have been reported in human adipose tissue (*9,10*), blood (*11,12*), breast milk (*13*), and serum (*14*). The purpose of this report, an extension of our previous studies (*8*), is to provide information about PBB distribution in human tissues and document its persistence in the body ten years after the initial contamination.

Materials and Methods

Specimens

Autopsied tissue samples (approximately 5 g) were obtained from subjects in the Grand Rapids area. The population of this area had previously been shown to have been exposed to relatively large amounts of PBB via the diet. Criteria for inclusion in the study were: (a) death was due to natural causes or accident (drug-related deaths were not included) and the subject was scheduled for autopsy, (b) a history of residency in the

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area during 1973, and (c) informed consent obtained from next of kin. Where possible, the following tissues were obtained from each of the 15 study subjects: adrenal, aorta, brain, left ventricle of the heart, kidney cortex and medulla, liver, lung, pancreas, perirenal fat, skeletal muscle, spleen, thymus, and thyroid. All samples were taken at autopsy and placed in PBB-free glass storage vials. The samples were stored frozen (–70°C) until analysis. Prior to analysis, all readily dissectable fatty tissue was removed from the specimens except, of course, for perirenal fat tissue. Samples were analyzed in order of receipt with all tissues of one subject completely analyzed before analysis of the next subject was started.

Quantitation of PBB

Hexane, isooctane, and petroleum ether were glass-distilled pesticide grade (Burdick and Jackson, Muskegon, MI). 60-100 mesh Florisil, PR grade, was obtained from the Floridin Co., Pittsburgh, PA; anhydrous sodium sulfate was obtained from Baker Chemical Co., Phillipsburg, NJ; acid-washed and ignited sand from Mallinckrodt, St. Louis, MO; and Firemaster BP-6 was from the FDA, Detroit, MI. All other chemicals and reagents wre reagent grade.

A Perkin-Elmer Sigma 1 gas chromatograph equipped with a ^{63}Ni electron capture detector, interfaced to a Perkin-Elmer Sigma 10 data station (Perkin-Elmer Corporation, Norwalk, CT) was used to quantitate PBB. The data system integrated the area under the peaks and provided a print-out of the concentration of the PBB isomers. The 1.8 m × 44 mm i.d. glass column was packed with 2% OV-1 on Gas Chrom Q (Supelco, Belfonte, PA). The carrier gas was argon-methane (95:5), and the flow rate was 50 mL/min. Prior to use, the column was conditioned for 24 hr at 270°C with carrier gas flowing. During chromatography the column temperature was 245°C, the injector temperature was 260°C, and the detector temperature was 320°C.

Extraction of PBB from Tissue

A modification of the procedures (*15,16*) previously used in our labortories is outlined here. Approximately 0.5 g of wet tissue was weighed and transferred to a 150-mL stainless steel beaker. The tissue was then ground with 5 g of sand and 5 g of anhydrous sodium sulfate, using a stainless steel rod. After the addition of 20 mL of hexane to the beaker, the beaker was warmed for 5 min in a water bath maintained at 70°C. The solution/slurry was filtered through Whatman No. 1 filter paper into a 35-mL test tube. This extraction procedure was repeated four times. At the end of each extraction, the hexane was evaporated under nitrogen at 70°C so that all of the extracts were concentrated in the same test tube. The funnel and filter paper were also rinsed an additional two times and these washings were added to the same tube and combined with the concentrated extracts. The entire extraction volume (110 mL) was concentrated to approximately 1 mL.

The concentrated extract was chromatographed on a 30 × 0.6 cm glass column containing 3.5 g Florisil. The top of the column was packed with a 2-cm length of anhydrous granular sodium sulfate. The column was preconditioned by washing with 10 mL hexane; the tissue extract was then added. The test tube was rinsed four times with 1 mL of hexane, the washes were sequentially added to the column, and chromatography was conducted with hexane as the mobile phase. The filtrate (12–15 mL) was collected in a clean 10 × 165 mm test tube, evaporated to dryness at 70°C under nitrogen, and dissolved in a known volume of hexane (2–10 mL, depending on amount of residue and initial sample weight); a 2 μL aliquot was injected onto the gas chromatograph column.

Quantitation of PBB was based on the amount of the 2,4,5,2′,4′,5′-hexabromobiphenyl peak (the most abundant component of Firemaster BP-6). A standard PBB curve was constructed of PBB concentration (ng/mL) versus area under the hexabromobiphenyl peak. The curve was linear from 0.5 to 1000 ng/mL PBB. Samples that had a concentration initially determined to be higher than 1000 ng/mL were appropriately diluted with hexane and re-analyzed. A prepared control was also analyzed after every 10 samples to assure the quality control of the procedure.

Results

A summary of pertinent subject information is presented in Table 1. The ages ranged from 18 to 84 years, with seven females and eight males. No attempt was made to correlate cause of death with PBB exposure or PBB tissue content. The concentration of PBB found in each tissue is presented in Table 2. The units were nanograms per gram of tissue, wet weight. Individual specimens not received are indicated by a dash. This information is also presented graphically in Figure 1. Quality control results are listed in Table 3. Pooled serum standards were prepared to give a final concentration

Table 1. Subjects used in PBB study.

No.	Sex	Age	Cause of death	County of residence
1	F	29	Pulmonary edema	Kent
2	M	25	Not known	Kent
3	F	72	Cerebral accident	Kent
4	F	37	Ruptured spleen	Kent
5	M	65	Exanguination	Muskegon
6	M	77	Acute M.I.	Kent
7	M	69	Abdominal hemorrhage	Kent
8	F	77	Carcinoma of the brain	Manistee
9	M	84	Acute M.I.	Ottawa
10	M	77	Acute M.I.	Kent
11	F	18	CO poisoning	Kent
12	M	59	Bilateral pneumonia	Ogemaw
13	F	71	Hepatoma	Manistee
14	F	57	Cardiac failure	Kent
15	M	82	M.I.	Kent

Table 2. PBB post-mortem study.

Subject no.	PBB in tissue, ng/g wet weight													
				Heart	Kidney					Renal	Skeletal			
	Adrenal	Aorta	Brain	l. vent.	Cortex	Medulla	Liver	Lung	Pancreas	fat	muscle	Spleen	Thymus	Thyroid
1	40	—	2	6	2	4	9	2	4	80	8	3	—	3
2	407	118	1	15	4	15	17	2	43	457	10	9	295	9
3	242	247	16	21	18	61	0	63	51	320	42	2	—	80
4	148	294	103	36	32	30	147	73	653	430	84	123	—	—
5	43	18	3	4	3	6	39	4	135	134	7	1	—	5
6	98	64	24	126	53	77	104	14	130	110	45	64	29	12
7	35	6	1	52	5	6	5	2	9	32	2	2	0	5
8	868	1011	142	233	61	100	259	93	188	1650	22	69	—	—
9	—	75	16	15	6	2	59	6	158	94	11	0	—	12
10	110	285	—	110	95	81	31	62	170	1110	57	312	—	40
11	602	107	0	21	29	38	37	11	148	607	33	15	617	41
12	196	29	11	4	17	31	30	80	92	322	13	4	30	22
13	17	44	9	4	2	3	22	4	20	205	14	2	—	6
14	48	—	5	6	10	5	15	4	17	167	8	4	—	8
15	850	514	13	37	42	67	143	43	146	1390	53	36	—	37
Mean	265	216	27	46	25	35	61	31	131	475	27	43	243	22
±SEM	80	77	12	16	7	9	18	9	41	131	6	21	140	6
Range	17–850	6–1011	1–103	4–233	2–95	2–100	0–259	2–93	4–653	32–1650	2–84	0–312	29–617	3–80
Tissue/ renal fat: mean ratio	0.56	0.45	0.06	0.10	0.05	0.07	0.15	0.07	0.28	1.0	0.06	0.09	0.51	0.06

Table 3. PBB quality control analysis

Sample no.	Concentration, ng/mL
1	26.8
2	33.4
3	27.2
4	31.2
5	29.7
6	27.4
7	30.8
8	27.9
9	26.5
10	28.2
11	26.1
12	32.2
13	30.5
14	32.0
15	30.7
16	24.8
17	24.9
18	31.9
19	27.0
20	29.3
21	28.0
22	33.0
23	25.4
24	27.6
25	27.3
26	25.6
27	32.5
28	26.4
Mean	28.73
SD	2.65
SEM	0.50

of 30 ng/mL with an analytically determined mean ± S.D. of 28.75 ± 2.65. These quality control samples related to reproducibility of the assay but cannot be directly related to recovery from the tissue samples. It should also be pointed out that there is no available information concerning recovery of PBB from tissues studied in this report using the described extraction technique. Since PBB is only moderately soluble in hexane, the values reported here are probably lower than what is actually present.

Renal fat, as expected, had the highest single PBB concentration (1650 ng/g) as well as the highest mean tissue concentration (475 ng/g). The other tissues can be grouped into three classes on the basis of PBB concentration relative to that of fat: high (ratios of 0.45-0.56; adrenal, atheromatus aorta, and thymus), medium (ratios of 0.1-0.28; pancreas, liver, left ventricle of the heart), and low (ratios of 0.02 to 0.09; kidney, lung, brain, skeletal muscle, thyroid and nonatheromatus aorta).

Discussion

The data presented here document, qualitatively, the persistence of PBB in human tissue for ten years. They also indicate that PBB is not confined to fat or fat-rich tissue but also appears to be distributed throughout the entire human body. As mentioned previously, no attempt was made to correlate PBB levels with cause of death or reported adverse health in each subject. Indeed, the question of potential long term adverse health effects associated with PBB remains unanswered.

It is of interest to speculate about the time course of PBB elimination from fat samples. This speculation is possible by comparing the data in our study with data obtained in an earlier study (*2*). The earlier study was conducted in 1978 on materials from live subjects, as compared to the present post-mortem study. The adi-

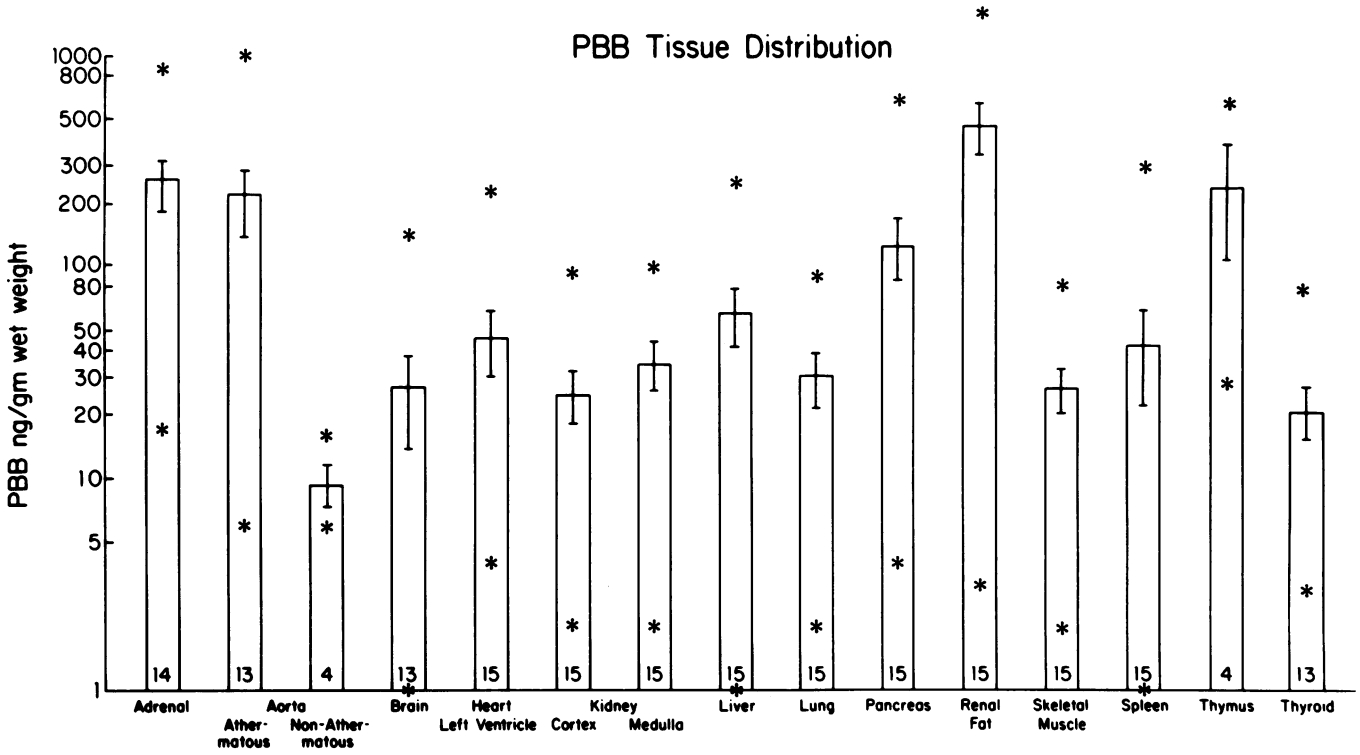

FIGURE 1. PBB concentrations (ng/g wet weight) in analyzed tissues. The top of the bar indicates the mean value ± SEM, the asterisks denote the range of values determined for each tissue, and the number of each individual tissue analyzed is within each bar.

pose tissue samples obtained in our study were obtained from the perirenal area whereas the adipose tissue samples of the previous study were obtained primarily from the gluteal and stomach areas. Unfortunately, there is no information about PBB concentration relationships in adipose tissue obtained from different sites of the body. For simplicity, we have assumed a 1:1 PBB concentration correspondence between these fatty tissue sources.

The mean statewide adipose PBB concentration reported (*2*) for 844 individual tissue sample analyses was 400 ng/g. The mean perirenal adipose PBB level reported in the present study was 475 ng/g. If these two values are compared, there is an apparent increase in PBB adipose tissue concentration in post-mortem samples. This could be due to a shift of PBB from other tissues to fat as we observed in rats (*8*). However, due to the wide range of PBB values observed in the previous study (*2*) and in our study, the difference of the mean is probably not significant and at best it appears that there was no major decline of PBB fat concentration. It is also likely that an inappropriate comparison of the mean data lead to these results, probably due to an inappropriate population comparison.

Most, but not all, of the residents in our study had a history of residency in the Grand Rapids area. This area encompassed both Kent and Muskegon Counties and is in the same location as the Muskegon County area of the previous study (*2*). This area constituted a "high" exposure area. Although a mean value from that area was not reported from that study, a median PBB value in adipose tissue was reported as 500 ng/g. The median PBB concentration in perirenal fat from our study was 320 ng/g tissue. Therefore, comparison of these numbers allows one to make an approximate estimation of the PBB adipose elimination half time ($t_{1/2}$). It must be cautioned, however, that the following discussion is speculation due to the large number of asdsumptions.

If one assumes that the initial median PBB adipose concentration was 500 ng/g approximately five years after the poisoning occurred and the second median PBB adipose concentration ws 320 ng/g approximately ten years after the poisoning occurred, then an estimated PBB adipose $t_{1/2}$) of 7.8 years can be calculated. This is substantially longer than the previously suggested PBB elimination half-time of ten months (*17*). Indeed, that report contains no data at all concerning the $t_{1/2}$). We are aware of no evidence documenting or supporting such a short half-time. If the 7.8 year estimate is approximately correct, it will take nine half-lives (70.2 years) for PBB spontaneously to reach a fat concentration of 1 ng/g. Support for this estimated half-life is provided by Tuey and Matthews (*18*). Using the pharmacokinetic data obtained from rats, these authors calculated an estimated body burden half-time in man of 6.5 years. That prediction is close to the estimation presented in this report.

It would be of importance to continue to obtain such data so that greater reliability in the median values can be obtained. It would also be of interest and importance to determine PBB concentrations in residents from a low exposure area and in individuals that have moved

into the State after the initial poisoning. This would determine if PBB is still entering or present in the food chain. The currently accepted view is that PBB no longer is entering the food chain. This, however, has never been documented.

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REFERENCES

1. Meester, W. D., and McCoy, D. J. Human toxicology of polybrominated biphenyls. In: Management of the Poisoned Patient (B. H. Rumack and A. R. Temple, Eds.), Science Press, Princeton, 1977, pp. 32–61.
2. Wolff, M. S., Anderson, H. A., and Selikoff, I. J. Human tissue burdens of halogenated aromatic chemicals in Michigan. J. Am. Med. Assoc. 247 (15): 2112–2116 (1982).
3. Kasza, L., and Weinberger, L. Comparative toxicity of PCB and PBB in the rat liver: Light and electron microscopic alterations after subacute dietary exposure. J. Environ. Pathol. Toxicol. 1: 241–257 (1978).
4. Tilson, H. A., and Cabe, P. A. Studies on the neurobehavioral effects of PBB in rats. Ann. N. Y. Acad. Sci. 320: 325–336 (1979).
5. Luster, M. I., Boorman, G. A., Harris, M. W., and Moore, J. A. Laboratory studies on PBB induced immune alterations following low level chronic or pre/post-natal exposure. Int. J. Immunopharmacol. 2: 69–80 (1980).
6. Kimbrough, R. C., Groce, D. F., Korver, M. P., and Burse, V. W. Induction of liver tumors in female Sherman strain rats by PBB. J. Natl. Cancer Inst. 66: 535–542 (1981).
7. Gupta, B. N., McConnell, E. E., Moore, J. A., and Haseman, J. K. Effects of a polybrominated biphenyl mixture in the rat and mouse. II. Lifetime study. Toxicol. Appl. Pharmacol. 68: 19–35 (1983).
8. Miceli, J. N., and Marks, B. H. Tissue distribution and elimination kinetics of PBB from rat tissue. Toxicol. Letters 9: 315–320 (1981).
9. Daum, S. M., Knittle, J., Rosenman, K., Rom, W. N., and Holstein, E. C. A simple technique for fat biopsy of PBB-exposed individuals. Environ. Health Perspect. 23: 183–185 (1983).
10. Meester, W. D., and McCoy, D. J. Human toxicology of polybrominated biphenyls. Clin. Toxicol. 10: 474 (1977).
11. Anderson, H. A., Wolff, M. S., Fischbein, A., and Selikoff, I. J. Investigation of the health status of Michigan Chemical Corporation employees. Environ. Health Perspect. 23: 187–191 (1978).
12. Anderson, H. A., Rosenman, K. D., and Snyder, J. Carcinoembryonic antigen (CEA) plasma levels in Michigan and Wisconsin dairy farmers. Environ. Health Perspect. 23: 193–198 (1978).
13. Brilliant, L. B., Van Amburg, G., Isbister, J., Humphrey, H., Wilcox, K., Eyster, J., Bloomer, A. W. and Price, H. Breastmilk monitoring to measure Michigan's contamination with polybrominated biphenyls. Lancet ii: 643–646 (1978).
14. Kreiss, K., Roberts, C., and Humphrey, H.E.B. Serial PBB levels, PCB levels and clinical chemistries in Michigan's PBB cohort. Arch. Environ. Health 37: 141–147 (1982).
15. Burse, V. W., Needham, L. L., Liddle, J. A., Bayse, D. D., and Price, H. A. Interlaboratory comprison for results of analyses for polybrominated biphenyls in human serum. J. Anal. Toxicol. 4: 22–26 (1980).
16. Price, H. A. Analytical procedure for PBB in adipose tissue. Michigan Dept. Publ. Health., internal document.
17. Isbister, J. I. PBBs in human health. Clin. Med. 83: 22–24 (1977).
18. Tuey, D. B., and Matthews, H. B. Distribution and excretion of 2,2′,4,4′,5,5′-hexabromobiphenyl in rats and man: pharmacokinetic model predictions. Toxicol. Appl. Pharmacol. 53: 420–431 (1980).